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SPECIAL REPORT

Regional haemodynamic changes during oral ingestion of N^G-monomethyl-L-arginine or N^G-nitro-L-arginine methyl ester in conscious Brattleboro rats

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Homozygous Brattleboro (i.e. vasopressin-deficient) rats were chronically instrumented with pulsed Doppler probes and intravascular catheters to permit continuous monitoring of regional haemodynamics. Over a 9 h period, rats drinking water showed no systematic changes in heart rate or mean arterial blood pressure although renal, mesenteric and hindquarters vascular conductances fell. These changes showed diurnal rhythyms, probably related to the nocturnal habits of rats. In separate groups of animals spontaneous oral ingestion of N^G-monomethyl-L-arginine (L-NMMA; 1 mg ml^{-1}) or N^G-nitro-L-arginine methyl ester (L-NAME; 0.1 mg ml^{-1}) caused marked hypertension but no significant bradycardia. Compared to control animals, rats drinking L-NMMA for 9 h showed significantly greater mesenteric and hindquarters vasoconstrictions, and rats drinking L-NAME showed greater vasoconstrictions in all 3 vascular beds.

Introduction Endothelial cell nitric oxide production is involved in the regulation of blood flow and pressure in mammals, including man (Vallance *et al.*, 1989; Gardiner *et al.*, 1990a; see Moncada *et al.*, 1989), and inhibition of nitric oxide synthesis by N^G-monomethyl-L-arginine (L-NMMA) leads to vasoconstriction and systemic hypertension (see Moncada *et al.*, 1989; Gardiner *et al.*, 1990a). Intravenous (i.v.) administration of bolus doses of L-NMMA in conscious, Long Evans rats causes marked decreases in regional vascular conductances that can be attenuated, to variable extents, by L- but not by D-arginine (Gardiner *et al.*, 1990a). These observations raise interesting questions about the long-term cardiovascular effects of prolonged inhibition of nitric oxide production.

Although techniques are available for chronic i.v. administration of drugs in rats, they are not without problems or expense. An attractive alternative would be a system in which prolonged inhibition of nitric oxide production could be achieved by spontaneous ingestion of an orally active compound, but normal rats drink relatively small volumes (20–30 ml 24 h^{-1}), with most drinking occurring during the dark period in association with eating; hence addition of substances to the drinking water might not achieve effective dosing. However, homozygous Brattleboro (i.e. vasopressin-deficient) rats have marked and continuous polydipsia (fluid intake 250–350 ml 24 h^{-1}). Therefore, these animals are a good test system, since their continuous drinking means that oral drug intake is relatively constant and allows low concentrations in the drinking water to be effective. In pilot experiments we determined that Brattleboro rats responded to i.v. L-NMMA in a similar way to Long Evans rats, and then investigated regional haemodynamic changes in Brattleboro rats drinking water that contained L-NMMA (1 mg ml^{-1}).

Recently it has been found that N^G-nitro-L-arginine is a more potent inhibitor of endothelium-dependent vasodilatation than is L-NMMA *in vitro* (Moore *et al.*, 1990). However, N^G-nitro-L-arginine is not readily soluble in water, whereas N^G-nitro-L-arginine methyl ester (L-NAME) is easily dissolved. Therefore, we extended the present study by also investigating the regional haemodynamic changes in Brattleboro rats drinking a solution of L-NAME at a concentration of 0.1 mg ml^{-1} since preliminary experiments showed that i.v. L-NAME had similar effects to i.v. L-NMMA, but was about 10 fold more potent. A preliminary account of some of this work has been given (Gardiner *et al.*, 1990b).

Methods Male, homozygous Brattleboro rats (350–450 g), bred in the Animal Unit at Nottingham, were anaesthetized (sodium methohexitone 60 mg kg^{-1}, i.p., supplemented as required) and had pulsed Doppler probes (Haywood *et al.*, 1981) sutured around left renal and superior mesenteric arteries and the distal abdominal aorta, as described previously (Gardiner *et al.*, 1990a). Animals were given an i.m. injection of 7 mg kg^{-1} ampicillin (Penbritin, Beechams Ltd) and returned to their home cages with free access to food and water. Between 7–14 days later animals were re-anaesthetized for the implantation of intravascular catheters; experiments were begun the following day when animals were fully conscious and unrestrained. Continuous recordings were made of mean blood pressure, instantaneous heart rate, and renal, mesenteric and hindquarters Doppler shifts signals (Crystal Biotech VF-1 system operating at a pulse repetition frequency of 125 kHz, and using HVPD-20 modules; Crystal Biotech, Holliston, MA, U.S.A.). Percentage changes in the mean Doppler shift signals are a good index of changes in volume flow (Haywood *et al.*, 1981) and percentage changes in conductances were calculated from mean Doppler shift and mean blood pressure signals (Gardiner *et al.*, 1990a).

Animals were separated into three groups: those drinking water ($n = 8$); those drinking L-NMMA (1 mg ml^{-1}, $n = 8$) and those drinking L-NAME (0.1 mg ml^{-1}, $n = 8$).

Baseline recordings were made between 06 h 30 min and 07 h 00 min and then, in groups 2 and 3, the drinking water was replaced with the solution of L-NMMA or L-NAME, respectively. Hourly intakes of water, L-NMMA or L-NAME and cardiovascular variables were recorded over the following 9 h. At each time point average values for the variables were taken over periods of about 5 min.

L-NAME methyl ester hydrochloride was obtained from Sigma; L-NMMA acetate was synthesized at Wellcome Research Laboratories (Beckenham).

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Within-group analysis of data was by Friedman's test comparing all values to the baseline state. Inter-group comparisons were made using the Kruskal-Wallis Test. A P value < 0.05 was taken as significant.

Results In Brattleboro rats drinking water the mean fluid intake was $9.0 \pm 0.4\,ml\,h^{-1}$ ($n = 8$) over 9 h. Heart rate and mean arterial blood pressure showed no systematic changes, although there were reductions in renal, mesenteric and hindquarters vascular conductances (Table 1). These changes in vascular conductances showed diurnal rhythms, probably related to the noctural eating habits of the rats.

The mean fluid intake of rats drinking L-NMMA was $8.8 \pm 0.5\,ml\,h^{-1}$ ($n = 8$). These animals showed marked increases in mean arterial blood pressure but no significant, sustained bradycardia (Table 1). By the end of the 9 h observation period there were decreases in mesenteric and hindquarters vascular conductances that were significantly greater than those seen in the control animals (Table 1).

Rats given L-NAME drank $8.5 \pm 0.8\,ml\,h^{-1}$ ($n = 8$) and showed marked hypertension but no significant bradycardia; there were renal, mesenteric and hindquarters vasoconstrictions (Table 1). In all 3 vascular beds the changes in conductances were greater than those seen in control rats and after 6 and 9 h, the hindquarters vasoconstriction was greater also than that seen in animals drinking L-NMMA (Table 1). Three of the animals drinking L-NAME died over-night; there were no deaths in the control or L-NMMA groups.

Discussion The present work in Brattleboro rats has shown that spontaneous oral ingestion of L-NMMA or L-NAME caused (within 1 h) hypertension which was sustained for the 9 h observation period. The hypertension was not accompanied by consistent bradycardia. Present evidence indicates that L-NMMA and L-NAME are potent inhibitors of endothelium-dependent nitric oxide synthesis and vasodilatation (Rees *et al.*, 1989; Moore *et al.*, 1990), but these compounds would be expected also to inhibit many other processes dependent upon nitric oxide production (Moncada *et al.*, 1989). Thus, although it is likely that the hypertension and increased vasoconstriction during L-NMMA or L-NAME ingestion were due, in part, to inhibition of vascular endothelial cell nitric oxide production we cannot exclude contributions from other mechanisms. Indeed, the absence of sustained bradycardia in the presence of hypertension indicates that cardiac baroreflex mechanisms, which in our experience are intact in Brattleboro rats (Gardiner & Bennett, 1988), were not operating normally in the presence of L-NMMA or L-NAME. Since there is evidence that nitric oxide might be involved in nociceptor afferent signalling (Duarte *et al.*, 1990), it is feasible that baroreceptor afferents might utilize a similar system and hence be influenced by L-NMMA or L-NAME. If baroreflex control of vasomotor efferent outflow was also blunted under these conditions this would have exacerbated the hypertension. These results contrast with those following acute i.v. injection of L-NMMA or L-NAME when the hypertension is accompanied by bradycardia in Long Evans rats (Gardiner *et al.*, 1989; 1990a,c) and in Brattleboro rats (unpublished observations).

By the end of the 9 h observation period the hypertension seen in rats drinking L-NMMA or L-NAME was associated with particularly marked hindquarters vasoconstriction; indeed, only in this vascular bed was flow reduced below baseline. Since, following acute i.v. administration of L-NMMA (Gardiner *et al.*, 1990a) or L-NAME (Gardiner *et al.*, 1990c) both renal and mesenteric vascular beds initially show substantial and greater vasoconstrictions than the hindquarters, the present results indicate the former two vascular beds are able to adjust for the inhibition of nitric oxide-mediated mechanisms in a way not possible in the hindquarters.

Although rats drinking L-NAME showed more marked hindquarters vasoconstriction than those drinking L-NMMA, both groups showed similar degrees of hypertension. Thus, it is probable that rats drinking L-NAME had a greater reduction in cardiac function than those drinking L-NMMA.

Table 1 Cardiovascular changes in Brattleboro rats drinking water (control), or solutions of N^G-monomethyl-L-arginine (L-NMMA; $1\,mg\,ml^{-1}$) or N^G-nitro-L-arginine methyl ester (L-NAME; $0.1\,mg\,ml^{-1}$)

Time (h)		1	3	6	9
Heart rate (beats min^{-1})	Control	-10 ± 6	4 ± 7	7 ± 12	5 ± 11
	L-NMMA	-11 ± 3*	-10 ± 7	-6 ± 6	-8 ± 7
	L-NAME	-25 ± 12	-20 ± 14	4 ± 8	1 ± 9
Mean blood pressure (mmHg)	Control	1 ± 1	4 ± 2	3 ± 3	-1 ± 1
	L-NMMA	13 ± 3*†	23 ± 6*†	23 ± 4*†	28 ± 7*†
	L-NAME	19 ± 6*†	33 ± 3*†	28 ± 2*†	32 ± 5*†
Renal flow (%)	Control	4 ± 5	1 ± 4	-3 ± 7	-13 ± 3*
	L-NMMA	15 ± 6°	6 ± 4	1 ± 7	1 ± 7
	L-NAME	-3 ± 4	-7 ± 7	-14 ± 7	-10 ± 7
Mesenteric flow (%)	Control	-15 ± 4*	-14 ± 3*	-18 ± 4*	-9 ± 6
	L-NMMA	-11 ± 3*	-19 ± 4*	-14 ± 5*	-13 ± 7
	L-NAME	-19 ± 5*	-26 ± 5*	-25 ± 6*	-12 ± 8
Hindquarters flow (%)	Control	-4 ± 5	-5 ± 6	-15 ± 4*	-18 ± 3*
	L-NMMA	-18 ± 3*	-31 ± 6*†	-33 ± 6*	-37 ± 6*
	L-NAME	-4 ± 5	-37 ± 4*†	-50 ± 2*†	-55 ± 4*†
Renal conductance (%)	Control	4 ± 5	-2 ± 4	-6 ± 6	-12 ± 3*
	L-NMMA	4 ± 4	-10 ± 5	-16 ± 6*	-18 ± 7*
	L-NAME	-17 ± 4*°	-29 ± 6*†	-32 ± 5*†	-31 ± 4*†
Mesenteric conductance (%)	Control	-16 ± 4*	-16 ± 4*	-19 ± 5*	-8 ± 6
	L-NMMA	-20 ± 3*	-32 ± 4*	-27 ± 5*	-28 ± 6*†
	L-NAME	-29 ± 3*†	-43 ± 4*†	-41 ± 5*†	-31 ± 7*†
Hindquarters conductance (%)	Control	-4 ± 6	-8 ± 7	-16 ± 6*	-18 ± 3*
	L-NMMA	-26 ± 2*†	-41 ± 6*†	-44 ± 6*	-48 ± 6*†
	L-NAME	-17 ± 7*	-51 ± 3*†	-60 ± 1*†°	-65 ± 4*†°

Values are mean ± s.e.mean, $n = 8$ for all the groups.
* $P < 0.05$ versus baseline (Friedman's test); † $P < 0.05$ versus control at same time (Kruskal-Wallis test); ° $P < 0.05$ L-NMMA versus L-NAME at same time.

It remains to be determined if direct or indirect cardiac effects, or both, of L-NAME were responsible for the deaths in this group.

We conclude that the experimental model used in the present work will be a useful tool in the investigation of the roles of nitric oxide in cardiovascular function in normal and pathophysiological states.

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